Evidence for Linkage Between Essential Hypertension and a Putative Locus on Human Chromosome 17

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Abstract—Several clinical and animal studies indicate that essential hypertension is inherited as a multifactorial trait with a significant genetic and environmental component. In the stroke-prone spontaneously hypertensive rat model, investigators have found evidence for linkage to blood pressure regulatory genes (quantitative trait loci) on rat chromosomes 2, 10, and X. In 1 human study of French and UK sib pairs, evidence for linkage has been reported to human chromosome 17q, the syntenic region of the rat chromosome 10 quantitative trait loci (QTL). Our study confirms this linkage \( (P=0.0005) \) and refines the location of the blood pressure QTL. \( \textit{(Hypertension. 1999;34:4-7.)} \)

Key Words: hypertension, essential \( \text{linkage} \) chromosome 17 \( \text{blood pressure} \) obesity

Essential hypertension is one of the most common cardiovascular diseases, affecting 15% to 20% of the population. It is an important risk factor for heart and kidney failure, myocardial ischemia, and stroke.\(^1\) Essential hypertension is considered a complex disease with significant genetic and environmental components that interact to play a role in blood pressure variation.\(^2\) Many candidate genes have been reported to contribute to the susceptibility to hypertension in clinical studies.\(^3\)–\(^5\)

The stroke-prone spontaneously hypertensive rat and the Dahl salt-sensitive hypertensive rat have proved to be useful tools in identifying quantitative trait loci (QTL) that contribute to blood pressure variations.\(^6\)–\(^11\) Evidence of a blood pressure QTL was found on rat chromosomes 2,\(^10\) 10,\(^6\)–\(^11\) and X.\(^7\) The rat chromosome 10 QTL is located near the angiotensin-converting enzyme (ACE) locus. The ACE locus is an attractive candidate gene because of its role in the renin-angiotensin system and the association between several polymorphisms in the ACE locus with blood pressure levels in some studies.\(^12\)–\(^14\) although other studies failed to confirm this finding.\(^15\)–\(^17\)

Human chromosome 17 is syntenic with rat chromosome 10, and evidence for linkage between this blood pressure QTL has been reported in French/UK hypertensive sib pairs.\(^18\) These markers are 18 cM proximal to the ACE locus. In addition, linkage of pseudohypoaldosteronism type IIB (hypertension, hyperkalemia, and normal renal glomerular filtration) to this region has been reported in several families.\(^19\) In this study, we tested a series of microsatellite markers near this chromosome 17 blood pressure QTL in a collection of white and black sib pairs from the United States. Evidence of linkage was found in our collection of white sib pairs.

Methods

Probands were identified from hypertension clinics in Massachusetts and Texas. Extensive family histories, including medical history, risk factor information, ethnic background, and demographics, were taken. Boston Medical Center Institutional Review Board for Human Subjects approved this study, and all subjects gave informed consent. Blood samples were collected from 74 white patients and 97 of their affected sibs (125 sib pairs) and from 45 black patients and 67 of their affected sibs (89 sib pairs). Individuals were considered affected if their blood pressure was \( >140/90 \) mm Hg or they were receiving antihypertensive treatment. Clinical information included age at onset of hypertension, medications, coexisting conditions, and body mass index (BMI). Subjects with secondary hypertension were excluded. Peripheral blood was obtained from the subjects in accordance with institutional guidelines for human subjects, and DNA was isolated from blood leukocytes with the Pure-Gene System.

Five microsatellite markers (average heterozygosity, 0.7) spanning the chromosome 17 blood pressure QTL were tested (Figure). These markers (D17S946, D17S1814, D17S800, D17S934, and D17S941) span a 12-cM region. An additional marker (D17S789) adjacent to the ACE locus 18 cM distal was tested (Figure). A standard amplification reaction (10 \( \mu \)L) was performed on 40 ng genomic DNA and 1.0 \( \mu \)mol each primer. Cycling parameters consisted of 95°C for 5 minutes, followed by 35 cycles of 94°C (1 minute), 60°C (30 seconds), and 72°C (30 seconds). Before amplification, 1 primer was end labeled with \( ^{32} \)P with polynucleotide kinase (New England Biolabs) so that the amplification product could be visualized by autoradiography after application to a standard sequencing gel. Size standards were used in each gel to ensure accurate allele size determination.

Sib-pair analysis was carried out with the SIBPAL,\(^20\) MAPMAKER/SIBS,\(^21\) and GENEHUNTER\(^22\) programs. MAPMAKER/SIBS estimates the maximum likelihood values of the proportion of sibs sharing 0, 1, and 2 alleles identical by descent (IBD) and computes a maximum LOD score by comparing the likelihood of the

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observed data under the estimated allele-sharing proportions to the likelihood under mendelian segregation. In this setting, the minimum possible LOD score is 0. Proportions of allele sharing were estimated under the “possible triangle” constraint, and no assumptions about dominance were made. The option that adjusts by 2/n for nonindependent pairs, which arise when there are ≥2 sibs in a family, was used. SIBPAL estimates the proportion of alleles that the sib pair shares IBD and calculates an overall mean proportion of alleles IBD. Excess allele sharing is evaluated by a scoring function $S$ all was used to evaluate allele sharing among those affected in a nuclear family simultaneously, in contrast to pairwise comparisons. This scoring function is distributed as a $Z$ statistic, and standard tests of significance were applied.

The allele frequencies used in the sib-pair analysis were calculated from the observed data by use of the first sibling in each nuclear family or, for whites, by use of allele frequencies reported in Centre d’Etude du Pyloromisme Humain (CEPH) families (56 chromosomes). The Spearman rank sum correlation was used to compare the CEPH and calculated frequencies. Analyses were conducted separately for whites and blacks.

To evaluate linkage to obesity, the Haseman-Elston regression approach was implemented in SIBPAL. This statistic evaluates linkage in concordant affected, concordant unaffected, and discordant sib pairs simultaneously.

### Results

Clinical information collected from 283 hypertensive subjects is shown in Table 1. Both whites and blacks had been hypertensive or had been treated with antihypertensive therapy for an average of 18 years. Similar proportions of whites and blacks had developed secondary complications of hypertension, including kidney disease, coronary heart disease, and stroke. Blacks had significantly higher BMIs ($P=0.0001$) and prevalence of diabetes ($P=0.049$) than whites.

A series of microsatellite markers spanning the blood pressure QTL, located 64 cM from p-ter of chromosome 17, were tested in this cohort to evaluate their potential role of this region in hypertension (Table 2 and the Figure). Suggestive evidence of linkage was observed for marker D17S800 in whites ($P=0.0005$) and for the adjacent maker D17S1814 ($P=0.006$). In addition, no evidence of linkage to marker D17S789 was observed in the region near the ACE locus in whites ($P>0.5$). These probability values were derived from MAPMAKER/SIBS. Other methods were slightly more conservative (GENEHUNTER) or liberal (SIBPAL), but the conclusions were consistent with results of MAPMAKER/SIBS.

For our initial studies, allele frequencies reported in CEPH families were used in linkage analysis of whites. However, given the possibility that the CEPH frequencies may not accurately represent our cohort, we conducted an analysis using a more conservative frequency derived from 1 person in each family. Comparison of the CEPH allele frequencies and our estimates showed close agreement ($r=0.97$ to 0.74) for all but 2 markers, D17S800 ($r=0.58$) and D17S941 ($r=0.5$). For D17S800, there was a predominant allele in the CEPH data (frequency=$0.42$) that was less frequent in our sample (frequency=$0.22$). The most common allele in our data (frequency=$0.276$) was relatively rare in the CEPH data (frequency=$0.11$). D17S941 had 3 alleles that were roughly equal in CEPH, but in our sample, 1 allele predominated (frequency=$0.48$). The results (Table 3) show an overall reduction in probability value of the 2 markers showing linkage (D17S800 and D17S1814). However, significant evidence for linkage was still observed ($P=0.004$ for D17S800 and $P=0.025$ for D17S1814).

In our group of black sib pairs, we found no evidence of linkage between hypertension and any of the markers ($P>0.5$). Power was assessed through simulation studies by use of family structures representative of the present study (38 sibships of 2; 14 sibships of 3). A 4-allele marker perfectly linked ($\theta=0$) to a gene with a dominant mode of inheritance was modeled. Although genotypes were simulated for parents, they were not used in the power calculations. The results show that we have >95% power to detect linkage to a gene at the $P=0.05$ significance level assuming no genetic heterogeneity in the population and 65% power assuming 5% genetic heterogeneity (ie, this gene is contributing to hypertension in >95% of the individuals in the study). Power is reduced as genetic heterogeneity increases; power to detect linkage is 50% for heterogeneity levels >10%.

Because the average BMI in our patient population was high, we examined the possibility that the chromosome 17 blood pressure QTL could include a component related to

### TABLE 1. Description of Patient Population

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>Whites (n=171)</th>
<th>Blacks (n=112)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age, y</td>
<td>63.96±13.74</td>
<td>58.4±12.06</td>
<td>0.0007</td>
</tr>
<tr>
<td>Age at which hypertension diagnosed, y</td>
<td>45.4±14.11</td>
<td>39.37±12.53</td>
<td>0.0029</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.68±5.41</td>
<td>33.68±8.45</td>
<td>0.0001</td>
</tr>
<tr>
<td>Kidney disease, %</td>
<td>9.9</td>
<td>9.3</td>
<td>0.89</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>13.4</td>
<td>22.4</td>
<td>0.049</td>
</tr>
<tr>
<td>Coronary heart disease, %</td>
<td>14.5</td>
<td>14</td>
<td>0.9</td>
</tr>
<tr>
<td>Stroke, %</td>
<td>5.8</td>
<td>6.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Values are mean± SD when appropriate.
been shown, resulting in a blood pressure change exceeding
between rat chromosomes 2 and 10 blood pressure QTL has
and systolic pressures. Furthermore, an epistatic interaction
sodium loading but has little or no effect on baseline diastolic
QTL affects systolic and diastolic blood pressures after
pressure variation on rat chromosomes 2, 10, and X. In
rat have led to the identification of QTLs contributing to blood
Results of genome-wide scans in the stroke-prone, spontane-
Evidence of linkage was found in our cohort of white sib pairs.
Strongest evidence of linkage was found in a 5-cM interval
found in a collection of 518 French/UK affected sib pairs.
humans (chromosome 17) of the rat chromosome 10 QTL was
5
3 groups: concordant obese (n = 56), discordant obese (n = 23), and discordant obese (1 obese and 1 nonobese, n = 29). These groups were again subjected to linkage analysis (Table 4). For marker D17S800, significant excess allele sharing was observed for concordant obese (P = 0.04) and discordant obese (P = 0.0017) sib pairs. Results were not significant for concordant nonobese sib pairs; however, the sample size was less than the other 2 groups. Given the excess allele sharing in both concordant and discordant obese groups and the nonsignificant Haseman-Elston results (probability value), there is no evidence for an obesity gene at this location. This analysis was conducted only in whites because the numbers of black sib pairs were too small for meaningful statistical evaluation when stratified.

Discussion
Results of genome-wide scans in the stroke-prone, spontaneously hypertensive rat and the Dahl salt-sensitive hypertensive rat have led to the identification of QTLs contributing to blood pressure variation on rat chromosomes 2, 10, and X. In another study, evidence of linkage to the syntenic region in humans (chromosome 17) of the rat chromosome 10 QTL was found in a collection of 518 French/UK affected sib pairs. Strongest evidence of linkage was found in a 5-cM interval between D17S946 and D17S932. Results of our study confirm this finding and narrow the size of the candidate region. Evidence of linkage was found in our cohort of white sib pairs.

Hilbert et al demonstrated that the rat chromosome 10 QTL affects systolic and diastolic blood pressures after sodium loading but has little or no effect on baseline diastolic and systolic pressures. Furthermore, an epistatic interaction between rat chromosomes 2 and 10 blood pressure QTL has been shown, resulting in a blood pressure change exceeding that predicted by each QTL independently. Thus, examination of the syntenic region of the rat chromosome 2 blood pressure QTL in humans and the potential interaction with the human chromosome 17 QTL is compelling. In this study, we found 2 adjacent markers on chromosome 17 that show linkage with essential hypertension in a collection of white sib pairs. Linkage was observed when allele frequencies derived from the test sample or from published values were used, demonstrating the robustness of this finding in light of the sensitivity of the results to allele frequency. Furthermore, this region overlaps a previously reported essential hypertension locus, providing evidence that a gene at this locus contributes to elevated blood pressure. Surprisingly, only 2 markers (D17S1814 and D17S800), which are 0.7 cM apart, show linkage. Detection of linkage to a narrow region could be due to increased power when markers are close to a causative gene (recombination fraction = 0).

Pseudohypoaldosteronism type IIB has been mapped to the same region as the blood pressure QTL on human chromosome 17. This disorder is characterized by autosomal-dominant transmission, hyperkalemia, normal renal glomerular filtration, and hypertension and is corrected with thiazide diuretics. Thus, there are considerable phenotypic differences between essential hypertension and pseudohypoaldosteronism type IIB. It is unclear whether these disorders are related; the differing phenotype may be due to mutations in the same gene that affect protein function differently or mutations in 2 unrelated genes.

The ACE gene is located 18 cM proximal to the blood pressure QTL and does not appear to overlap the candidate region. ACE has been an attractive candidate gene for essential hypertension because of its role in the renin-angiotensin system. However, genetic association or linkage between the ACE locus and hypertension in humans has been reported in some studies but not in others. Other

<table>
<thead>
<tr>
<th>Locus</th>
<th>Distance From p-ters, cM</th>
<th>SIBPAL IBD Sharing</th>
<th>SIBPAL P</th>
<th>GENEHUNTER Information Content</th>
<th>GENEHUNTER LOD Score</th>
<th>MAPMAKER/SIBS LOD Score</th>
<th>MAPMAKER/SIBS P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D175946</td>
<td>61.0</td>
<td>0.5361</td>
<td>0.1028</td>
<td>0.1361</td>
<td>0.675</td>
<td>0.4023</td>
<td>0.15</td>
</tr>
<tr>
<td>D1751814</td>
<td>62.2</td>
<td>0.5587</td>
<td>0.0048</td>
<td>0.0448</td>
<td>0.668</td>
<td>1.1529</td>
<td>0.025</td>
</tr>
<tr>
<td>D175800</td>
<td>62.9</td>
<td>0.5765</td>
<td>0.0028</td>
<td>0.0167</td>
<td>0.683</td>
<td>1.7701</td>
<td>0.004</td>
</tr>
<tr>
<td>D175934</td>
<td>63.7</td>
<td>0.5029</td>
<td>0.4586</td>
<td>0.5594</td>
<td>0.728</td>
<td>0.0301</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>D175941</td>
<td>72.7</td>
<td>0.4978</td>
<td>0.5318</td>
<td>0.4073</td>
<td>0.638</td>
<td>0.3056</td>
<td>0.2</td>
</tr>
<tr>
<td>D175789</td>
<td>90.8</td>
<td>0.4156</td>
<td>0.9894</td>
<td>0.9891</td>
<td>0.770</td>
<td>0.0000</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>
potential candidate genes on this segment of chromosome 17 include the Cl-HCO₃ exchanger, phenylethanolamine N-methyltransferase, nerve growth factor receptor, and pseudohypoaldosteronism type IIB genes.¹⁸,¹⁹ Additional linkage studies in other ethnic populations are warranted to further narrow the candidate region and to provide additional support for a gene at this location.

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References
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